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VITAMIN B AND PHENOTHIAZINE ANAEMIA IN DOGS[1]

BY H. BRUCE COLLIER[2] AND GEORGE E. MACK, JR.[3]

Abstract

Phenothiazine caused a haemolytic anaemia in three dogs used in a total of eight experiments. In one animal a diet deficient in vitamin B greatly intensified the anaemia and jaundice. Addition of vitamin B complex to normal diets did not prevent the anaemia, but it did suppress Heinz body formation and stimulate reticulocytosis. It is indicated that the B complex is necessary for erythropoiesis, but that it may have no direct effect upon the anaemia.

One animal died after four courses of the drug totalling 340 gm.; its liver revealed a portal cirrhosis.

Phenothiazine has been used successfully as an anthelmintic in man and in domestic animals. Unfortunately, an acute haemolytic anaemia frequently appears, although there are noteworthy species differences in susceptibility to the drug. Anaemia in rats, rabbits, and humans has been described by DeEds and co-workers (25, 5); in horses by Swales, Collier, and Allen (23); and in dogs by Schnitzer, Siebenmann, and Bett (19). Collier and Allen (3) reported an acceleration of haemolysis by phenothiazine derivatives *in vitro*. Worthy of note is the fact that ruminants show no toxicity after very large doses of phenothiazine. Rabbits, guinea pigs, and golden hamsters were also found to show no significant blood changes (24).

In humans, as in horses, the susceptibility of some individuals to anaemia has constituted a serious drawback to the clinical use of phenothiazine. The death of a child in England (9) resulted in the warning that "[haemolysis] is so usual with the anthelmintic phenothiazine that it is unjustifiable to use it in man." (1).

In view of the discovery of Rhoads and co-workers (16, 17) that vitamin B deficiency intensified indole anaemia in dogs, it was thought desirable to investigate the possible relationship between the B complex and phenothiazine anaemia. Dogs were chosen for the experiments described below.

Experimental

Methods

Adult mongrel dogs were kept on a normal diet of Purina fox chow. Vitamin B deficiency was produced by substituting the Goldberger diet of Miller and

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Contribution from the Department of Biochemistry, Dalhousie University, Halifax, N.S., with financial assistance from the National Research Council of Canada.

[2] *Associate Professor.*

[3] *Graduate Student.*

Rhoads (14) modified as follows: the peas were omitted and the casein was increased from 10 to 12.5%; peanut oil was substituted for cottonseed oil. Commercial phenothiazine was suspended in 10% gum acacia solution, with a little bile salt as wetting agent, and was administered to the animals by stomach tube. In the hope of reducing the effect of individual variation, seven of the eight experiments were carried out on two dogs only, one being used in four experiments, the other, in three.

For the blood analyses venous blood was withdrawn and oxalated with the potassium–ammonium-oxalate mixture of Heller and Paul (8). Haemoglobin concentration was determined photoelectrically by the cyanmethaemoglobin method as described elsewhere (2). The precision of the erythrocyte counts was about ± 0.1 million per μl., based upon the estimated standard error of the mean count of 10 squares. Reticulocytes were counted by the method given by Kracke (10, p. 630). Total erythrocyte volume was determined in Wintrobe haematocrit tubes centrifuged for 20 min. at 3000 r.p.m. with a relative centrifugal force of 1800. Plasma bilirubin was estimated by the method of Malloy and Evelyn (13), the supernatant plasma from duplicate haematocrit tubes usually being employed for this purpose.

Erythrocyte fragility was measured by a modification of Singer's (21) lysolecithin haemolysis method; it was thought that this procedure might more nearly represent physiological haemolysis than the usual hypotonic method. The lysolecithin was that previously described (3) and its lytic action was estimated photoelectrically by the method of Wilbur and Collier (26). Fragility was expressed as micrograms of lysolecithin required to bring about 50% haemolysis of 10 ml. of 1 : 100 whole blood in 0.95% sodium chloride in 10 min. at 25° C. This value was believed to represent the net antihaemolytic power of the whole blood against lysolecithin, both erythrocytes and plasma being taken into account (3)*.

The results of the blood examinations are presented in tabular form. The pretreatment levels are the averages of values obtained over several days, together with their average deviations. The results of many days' examinations have been omitted from the tables, only the essential data being presented.

Expt. 1. Dog MBL, dosage 5 gm./kg. on normal diet

Only one experiment was carried out on this dog, a male weighing 11 kg. A total of 55 gm. of phenothiazine was given in five doses over six days. As seen in Table I a definite anaemia developed in about eight days. Heinz bodies appeared at the height of the anaemia. Nucleated erythrocytes and reticulocytes indicated active regeneration. Two bilirubin estimations pointed to a definite haemolytic icterus, although the normal level was not determined.

** In anaemic blood the ratio of cells to plasma is of course altered. Control tests on blood diluted with plasma indicated that the fragility was very slightly decreased under these conditions. Evidently the effect of reducing the cell concentration is more than compensated for by the greater antihaemolytic power of the plasma.*

TABLE I

EXPT. 1. DOG *MBL*, DOSAGE 5 GM./KG. ON NORMAL DIET*

Date	Treatment	Hb	R.B.C.	Vol.	MCHb	MCV	Retics.	W.B.C.	Bili-rubin	Heinz bodies	Frag-ility	Remarks
Feb. 18	11 gm. Pz	18.5 ±0.3	7.34 ±0.15	53.0 ±1.2	25.2	72	0.5	6.9		–	—	
19	11 gm. Pz											
20	11 gm. Pz											
22	11 gm. Pz	17.0	6.8	49	25.0	72	0.5	10.1		–	—	
23	11 gm. Pz											
24		13.4	5.2	39	25.7	75	2.0	9.1	3.2	–	—	
26		10.6	4.0	—	26.9	—	4.0	11.4	—	–	—	Nucleated *R.B.C.*
28		11.9	4.3	32	27.6	76	7.0	11.5	—	++	—	Nucleated *R.B.C.*
Mar. 2		10.9	4.4	36	24.8	82	25	12.5	—	+	—	Nucleated *R.B.C.*
4		11.7	3.9	41	30.0	105	10	6.8	2.0	–	—	
9		14.3	5.7	46	25.1	80	3.0	10.0	—	–	—	
25		16.9	—	—	—	—	—	—	—	–	—	

*NOTE: *Pretreatment values are averages ± average deviation.*
Pz = phenothiazine.
B = vitamin B complex parenteral.
Hb = haemoglobin concentration, in gm. per 100 ml.
R.B.C. = erythrocytes, in millions per μl.
Vol. = erythrocyte volume, by haematocrit, %.
MCHb = mean corpuscular haemoglobin, in μμg.
MCV = mean corpuscular volume, in μ^3.
Retics. = reticulocyte percentage.
W.B.C. = leucocytes, in thousands per μl.
Bilirubin = plasma bilirubin, in mg. per 100 ml.
Fragility = erythrocyte fragility, as μg. lysolecithin for 50% lysis of 10 ml. 1 : 100 blood.

Expt. 2. Dog FPG, dosage 5 gm./kg. on normal diet

Four experiments were carried out upon a female dog, weighing 20 kg. In this experiment 100 gm. of phenothiazine was administered in 10-gm. lots over 12 days. Complete analyses were not performed, but an anaemia was indicated by a drop in haemoglobin from 14.3 to 9.4 gm. per 100 ml. in 11 days. The leucocyte count increased from 10,000 to 20,000 per μl. A single determination of bilirubin on the 11th day gave a value of 6.2 mg. per 100 ml., showing that a marked haemolytic jaundice had developed.

Expt. 3. Dog FPG, dosage 5 gm./kg. on B-deficient diet

In the second experiment upon this animal she was placed on the Goldberger diet for 16 days previous to the administration of the drug. In this time there

was no loss in weight nor evidence of black tongue, but a decrease in appetite was observed. The phenothiazine totalling 100 gm. was given in 20-gm. lots over six days. The normal diet was restored on the 15th day from commencement of dosing, and was supplemented with liver extract and yeast concentrate in order to hasten recovery.

Table II shows that a very severe anaemia developed under these conditions, accompanied by an acute jaundice; the positive direct van den Bergh at the height of the jaundice suggested some degree of liver damage. Heinz bodies were very numerous and persisted for 11 days. Although reticulocytosis was not marked the animal returned to normal in just over one month.

TABLE II

EXPT. 3. DOG *FPG*, DOSAGE 5 GM./KG. ON B-DEFICIENT DIET

Date	Treatment	Hb	R.B.C.	Vol.	MCHb	MCV	Retics.	W.B.C.	Bili-rubin	Heinz bodies	Frag-ility	Remarks
Dec. 9	20 gm. Pz	14.7 ±0.4	5.98 ±0.28	42.7 ±1.6	24.6	72	—	9.7	1.0	—	—	
11	20 gm. Pz											
13	20 gm. Pz											
14	20 gm. Pz	12.9	5.80	36	22.2	62	—	26.8	2.7	—	—	
15	20 gm. Pz											
16		12.4	4.35	31	28.6	71	2.0	21.1	3.8	+++	—	
18		10.3	3.14	26	32.7	83	1.6	13.0	10.2	+++	—	
21		4.6	1.45	13	31.4	90	—	—	10.3	++	—	Direct van den Bergh 4.5
22	Liver extract	5.0	1.70	15	29.6	91	—	—	5.2	++	—	
26		8.7	2.92	28	30.2	97	4.0	13.2	3.9	+	—	
Jan. 2		12.5	4.59	39	27.3	85	4.0	5.1	2.3	—	—	
12	Yeast	13.6	5.84	44	23.4	77	<0.5	7.7	—	—	—	

Expt. 4. Dog FPG, dosage 5 gm./kg. on supplement of B complex

This experiment was carried out three months after the previous one. The normal diet was supplemented during administration of the drug by injections of Vitamin B Complex Parenteral, *Lederle*, totalling 5 ml. The phenothiazine, 100 gm., was administered over seven days and the results are given in Table III. It is apparent that the vitamin B supplement did not prevent the development of a moderate anaemia. Worthy of note, however, is the complete absence of Heinz bodies and the low level of bilirubin. Reticulocytosis was not marked but recovery was rapid. A definite increase in fragility was observed.

TABLE III

EXPT. 4. DOG *FPG*, DOSAGE 5 GM./KG. ON SUPPLEMENT OF B COMPLEX

Date	Treatment	Hb	R.B.C.	Vol.	MCHb	MCV	Retics.	W.B.C.	Bilirubin	Heinz bodies	Fragility	Remarks
Mar. 22	1 ml. B											
23	1 ml. B 20 gm. Pz	13.4 ±0.2	6.13 ±0.13	43.7 ±1.0	21.9	71	<0.5	9.0	0.30	—	220	
24	1 ml. B 20 gm. Pz											
Mar. 25	1 ml. B 20 gm. Pz	13.0	5.96	41.2	21.9	69	0.5	16.5	—	—	200	
27	1 ml. B 20 gm. Pz											
29	20 gm. Pz	10.8	4.94	—	21.9	—	8.0	15.0	1.30	—	170	
April 1		9.8	3.74	32.6	26.1	87	5.0	10.6	0.36	—	200	
6		13.5	5.62	44.3	23.9	79	2.0	7.9	0.26	—	205	
14		15.7	6.18	44.5	25.7	72	<0.5	7.4	—	—	220	

Expt. 5. Dog FPG, dosage 2 gm./kg. on supplement of B complex

In this final experiment upon the female, two months after the previous one, the dosage of the drug was reduced to 40 gm. and was accompanied by injections totalling 14 ml. of the Vitamin B Complex Parenteral. Table IV indicates that only a negligible drop in haemoglobin and in erythrocyte count was observed; since no other experiments were carried out at this dosage level, the effect of the B complex cannot be accurately assessed. As before, no Heinz bodies appeared. The most noteworthy finding was the bilirubinaemia, which could not be accounted for by the degree of erythrocyte destruction; the abnormally high pretreatment level of bilirubin suggested some liver damage.

One month after the completion of this experiment the dog died. She had received a total of 340 gm. of phenothiazine over a period of eight months. An autopsy was performed by Professors E. G. Young and C. B. Weld, who reported as follows:—

> The intestines were definitely cyanotic and the mesenteric and omentum fat was excessively yellow in colour. The mesenteric vessels were very prominent, distended, and varicosed; the anastomosis between the vessels of the portal and vena cava (inferior) systems was highly developed.
>
> The liver was very abnormal, nodular, and pigmented, with loss of the usual lobular configuration. The nodules were yellowish in colour. The spleen was normal, as were other organs by superficial examination.

An examination by Professor R. P. Smith of the Department of Pathology yielded the following information:—

> The liver reveals a portal cirrhosis of the healed yellow atrophy type with marked fatty infiltration of the liver cells in the nodular areas. Duodenum: there is a mild simple inflammatory change of its mucosa. Jejunum and ileum: show no special abnormal changes.

TABLE IV

EXPT. 5. DOG *FPG*, DOSAGE 2 GM./KG. ON SUPPLEMENT OF B COMPLEX

Date	Treatment	Hb	R.B.C.	Vol.	MCHb	MCV	Retics.	W.B.C.	Bili-rubin	Heinz bodies	Frag-ility	Remarks
May 21	2 ml. B											
June 1	2 ml. B											
5	2 ml. B 20 gm. Pz	13.2 ±0.1	5.20 ±0.09	39.5 ±1.0	25.4	76	<0.5	11.3	1.25	–	150	
7	2 ml. B 20 gm. Pz											
8	2 ml. B	12.8	4.85	36.8	26.5	76	<0.5	11.7	3.68	–	130	
10		13.0	5.00	38.3	26.0	77	2.0	10.3	4.43	–	—	
12		11.3	4.66	34.6	24.3	74	2.5	13.6	4.70	–	125	
14	2 ml. B	11.8	4.72	35.3	25.0	75	2.0	11.2	3.22	–	125	
16	2 ml. B	12.6	5.05	38.1	25.0	76	2.0	9.5	1.87	–	150	
28		14.0	5.67	41.6	24.7	73	—	11.4	0.89	–	150	
July 9	Died											

Expt. 6. Dog MPT, dosage 5 gm./kg. on supplement of fresh liver

This male animal weighed 20 kg. One hundred gm. of phenothiazine was given in six days and the normal diet was supplemented with 930 gm. of fresh beef liver before and during treatment. Table V shows that the anaemia was rather severe, accompanied by a few Heinz bodies. The reticulocyte response was good, the blood picture returning to normal in about six weeks. There was a slight degree of bilirubinaemia. A leucocytosis, with a shift to the left, accompanied the anaemia.

Expt. 7. Dog MPT, dosage 2.5 gm./kg. on B-deficient diet

The animal, two months after the previous experiment, was placed upon the Goldberger diet for four weeks; there were no symptoms of vitamin B deficiency other than slight loss of appetite. Fifty gm. of phenothiazine was administered in three days; the results are given in Table VI. The anaemia was less severe than in the previous experiment (with 5 gm./kg.), but the reticulocyte response was poor. Bilirubin levels remained very low. Leucocytosis was marked, the white cells in the haematocrit tubes forming two distinct layers of about equal depth.

Expt. 8. Dog MPT, dosage 2.5 gm./kg. on supplement of B complex

Three months after the last experiment the animal was subjected to 50 gm. of the drug, accompanied by injections of the Vitamin B Complex Parenteral totalling 12 ml. Table VII indicates that the anaemia was of about the same

TABLE V

EXPT. 6. DOG *MPT*, DOSAGE 5 GM./KG. ON SUPPLEMENT OF FRESH LIVER

Date	Treatment	Hb	R.B.C.	Vol.	MCHb	MCV	Retics.	W.B.C.	Bili-rubin	Heinz bodies	Frag-ility	Remarks
Feb. 22	200 gm. liver											
23	100 gm. liver											
24	75 gm. liver											
26	100 gm. liver, 20 gm. Pz	17.2 ±0.2	6.70 ±0.13	52.0 ±2.0	26.0	78	<0.5	8.4	0.30	–	—	
28	130 gm. liver, 20 gm. Pz	16.2	6.31	48	25.8	76	<0.5	24.5	—	–	—	
Mar. 1	125 gm. liver, 20 gm. Pz											
2	75 gm. liver 20 gm. Pz	13.1	5.13	38	25.7	75	<0.5	23.8	—	+	—	
3	125 gm. liver, 20 gm. Pz											
4		9.8	3.78	35.6	25.7	94	2.0	31.9	2.3	++	—	
6		10.5	3.36	29.3	31.4	87	20	28.0	—	–	—	Nucleated *R.B.C.*
9		10.3	3.35	32.0	30.6	96	10	14.6	0.5	–	—	Nucleated *R.B.C.*
16		13.0	5.48	43.1	23.7	78	1.0	11.0	—[1]	–	—	
April 6		17.7	6.83	50.7	25.9	74	<0.5	9.6	—	–	—	

TABLE VI

EXPT. 7. DOG *MPT*, DOSAGE 2.5 GM./KG. ON B-DEFICIENT DIET

Date	Treatment	Hb	R.B.C.	Vol.	MCHb	MCV	Retics.	W.B.C.	Bili-rubin	Heinz bodies	Frag-ility	Remarks
Mar. 24	B-free diet											
April 22	18 gm. Pz	18.2 ±0.5	7.07 ±0.17	52.1 ±1.8	25.3	73	<0.5	8.5	0.28	–	200	
23	15 gm. Pz											
24	17 gm. Pz	16.8	6.47	47.5	25.9	73	0.5	37.1	0.27	–	140	
26		15.9	6.35	47.2	25.0	74	0.5	10.6	0.19	+	140	
28		14.9	6.23	45.9	23.9	74	0.5	9.4	0.15	+	135	
30		14.2	5.72	44.5	24.8	78	0.5	8.2	0.17	+	150	
May 3		12.7	4.83	38.0	26.3	79	0.5	6.4	0.29	–	155	
10		13.6	6.25	42.4	21.8	68	0.5	8.5	0.23	–	195	
15		14.7	6.30	45.6	23.3	72	2.0	7.3	—	–	190	
22		15.9	6.56	49.5	24.2	75	<0.5	9.9	—	–	200	

TABLE VII

EXPT. 8. DOG *MPT*, DOSAGE 2.5 GM./KG. ON SUPPLEMENT OF B COMPLEX

Date	Treatment	Hb	R.B.C.	Vol.	MCHb	MCV	Retics.	W.B.C.	Bili-rubin	Heinz bodies	Frag-ility	Remarks
July 14	2 ml. B											
16	2 ml. B											
19	2 ml. B 20 gm. Pz	17.4 ±0.4	7.36 ±0.28	50.0 ±1.1	23.6	68	<0.5	—	0.35	—	195	
20	2 ml. B 20 gm. Pz											
21	2 ml. B 10 gm. Pz	16.2	6.62	47.3	24.3	71	—	—	0.79	—	90	
23		15.0	5.84	45.0	25.7	77	15	—	0.15	—	90	
26	2 ml. B	13.0	5.16	39.0	25.2	76	—	—	0.11	—	100	
30		11.6	4.27	35.5	27.2	83	2.0	—	—	—	135	
Aug. 4		13.3	4.86	40.2	27.4	83	2.0	—	—	—	170	
12		15.5	6.70	47.5	23.2	71	1.0	—	0.15	—	170	
24		17.1	6.34	50.0	27.0	79	—	—	—	—	160	

degree as in the previous experiment. Heinz bodies were completely absent, however. Reticulocytosis was not marked. The bilirubin level remained low. The erythrocyte fragility increased very sharply in this experiment, then returned slowly to normal.

Discussion

The Nature of the Anaemia

The anaemia caused by phenothiazine in dogs is of the same haemolytic type as observed in horses (23). Haemoglobin, erythrocyte count, and erythrocyte volume all decrease in parallel fashion. The bilirubinaemia commonly observed also indicates an abnormal destruction of red cells. That the anaemia is not due to impairment of erythropoiesis is evident from the reticulocytosis, which is apparently stimulated by the anaemia. The increase in mean corpuscular haemoglobin and volume that is observed during regeneration is presumably due to the fact that the immature cells that are being thrown into the circulation are larger than the mature erythrocytes.

A leucocytosis, primarily a neutrophilia, invariably accompanied the anaemia. This appears to be a feature common to various types of anaemia (10, p. 237).

In all experiments in which fragility was measured, an increase in fragility accompanied, and in fact preceded, the anaemia. This was doubtless due to the action of phenothiazine derivatives in accelerating haemolysis (3), an effect that may explain their haemolytic action.

The appearance of Heinz bodies is a feature of phenothiazine anaemia as observed by Swales, Collier, and Allen (23) in horses and by Siebenmann (20) in mice and monkeys. These small dense bodies attached to the erythrocytes are characteristic of many drug anaemias, and were first observed by Heinz (7). The formation of these bodies has been discussed by Cruz (4) and their chemistry by Kunkel (11) and Richardson (18). They have been found in the sulphonamide anaemias (15, 18). The relationship of Heinz bodies to red cell destruction is unknown, although Cruz refers to the cells with Heinz bodies as damaged erythrocytes, which are more fragile than normal cells.

The Effect of Vitamin B

Rhoads and co-workers (16, 17) found that indole was not haemolytic in dogs on a normal diet, whereas on a vitamin B-deficient diet an anaemia was readily produced. In the present series of experiments with phenothiazine the effect of the B complex is not so clear cut. For example, the male dog *MPT* exhibited about the same degree of anaemia when on a deficient diet as when on a diet supplemented with the B complex (Expt. 7 and 8). In the female animal *FPG*, on the other hand, vitamin B deficiency resulted in a very acute anaemia (Expt. 3). It was not possible to prevent the anaemia by addition of vitamin B to the normal diets, although the blood changes were negligible in one experiment on a reduced dosage of the drug (Expt. 4).

Erythrocyte fragility did not seem to be affected by the nature of the diet, suggesting that lack of vitamin B did not result in formation of abnormally fragile cells. The formation of Heinz bodies was suppressed, however, by the vitamin supplements; as pointed out above the relationship of these bodies to red cell destruction is obscure.

In most of the experiments reticulocytosis was apparently stimulated by adequate amounts of the B complex and was inhibited by a deficiency. This effect is probably the clue to the relationship between the B vitamins and the haemolytic anaemia. It seems probable that the B complex has no direct relation to the rate of erythrocyte destruction, but that its role is to maintain rapid regeneration of new cells. On a deficient diet, regeneration cannot keep pace with the increased rate of haemolysis due to the drug and a severe anaemia results.

The ruminants, which synthesize their own vitamin B, are not susceptible to phenothiazine anaemia, but it seems clear that other factors must contribute to the situation. The fragility of ruminant erythrocytes is notably low. Furthermore, the ability of the liver to detoxify phenothiazine may depend upon the availability of the B complex.

The concept that the role of the B vitamins in the drug anaemias is to stimulate erythropoiesis agrees with the hypothesis recently put forward that some as yet unidentified members of the B complex may play an essential role in the formation of new red cells. This is discussed by Williams in his review (27) and in several more recent papers (12, 22, 28). In the light of these

findings, it would seem advisable to administer adequate doses of the B complex in cases of drug anaemia.

Toxic Reactions

A possible objection to the plan of the investigation is that the same animals were used repeatedly. Although this was designed to minimize individual variation it was realized that either tolerance or increased sensitivity to the drug might be developed. It is tempting to suggest that the death of the female dog *FPG* was due to a toxic reaction occasioned by the administration of a large amount of phenothiazine over several months. It is possible that during the experiment on the B-deficient diet the liver, in a subnormal nutritional state, received some permanent injury. The bilirubinaemia observed prior to the last experiment (Expt. 5) supports this view. Fouts (6) has described a similar liver damage, accompanied by anaemia, in animals on a low protein diet containing only the synthetic B vitamins and lacking in the as yet unknown members of the B complex.

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STUDIES OF SHOCK PRODUCED BY MUSCLE TRAUMA

I. METHODS; MORTALITY; CARDIOVASCULAR, BLOOD CONCENTRATION, AND SUGAR CHANGES[1]

By R. A. Cleghorn[2] and A. D. McKelvey[3]

Abstract

A large series of dogs, subjected to severe muscle trauma, has been studied. Eighty-four per cent died or would have died of shock within 24 hr. had they not been treated with a blood substitute. Of these about half developed severe shock in less than five hours. A few, 3.7% of the series, died between 24 and 80 hr., and 12.3% were considered indefinite survivors either being well when killed at 24 hr. or appearing well at the end of three days after trauma.

Repeated observations made on the blood pressure and heart rate made it possible to predict death some time in advance in a great majority of cases. Haemoconcentration, as evidenced by an increase in the volume of packed red blood cells, occurred in the majority of the animals. This is ascribed partly to the fact that the fluid loss into the damaged tissues was principally plasma rather than whole blood, and partly to the fact that the animals were not deeply anaesthetized for a long time and consequently reflex splenic contraction added cells to the circulation. The blood sugar in dogs dying within three hours of trauma was normal or elevated. In dogs dying later it was often low. In those in which life was prolonged a few hours by a blood substitute the terminal value was very low. In many dogs in which life was prolonged beyond 24 hr. the blood sugar values slightly before death were within normal limits.

The significance of these findings is discussed.

It is now generally conceded that reduction in blood volume is the most important initiating cause of secondary shock but once the process has been started there may be sustaining factors. Toxins liberated from damaged tissue and inadequate response of the adrenal cortex have been so considered. Moon (16) has done much to rearouse interest in traumatic toxemia as a cause of shock. Selye (18) has focussed attention on the hypertrophy of the adrenal cortex that occurs following various forms of stress and that is thought to be a defensive reaction. In a critical review of adrenal cortical hypertrophy Tepperman, Engel, and Long (24) remark on the relation of the adrenal cortex to shock and describe lipoid depletion of that tissue as being an early sequel to haemorrhage. Activation of the adrenal cortex in shock is further suggested by observations of Weil and Browne (25) that show that an increased urinary excretion of cortin-like material occurs in injured people. Finally the work of Swingle *et al.* (21) has proven that adrenal steroids provide protection against shock, in adrenalectomized dogs at least.

The toxic and humoral aspects of shock may be related. It is possible that adrenal cortical hormones may be concerned in the metabolism of toxic

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Contribution from the Department of Medicine, University of Toronto, Toronto, Ont. The work was aided by a grant from the National Research Council.

[2] *Demonstrator, Department of Medicine, University of Toronto. Now on leave of absence with the Canadian Army overseas.*

[3] *Voluntary Assistant, Department of Medicine, University of Toronto.*

agents liberated from damaged tissues. Such a relationship is known to be true for potassium (14), which has been suggested by Scudder (17, 26) as a toxic factor in shock. This ion has been shown by Manery and Solandt (15) to be liberated from experimentally traumatized muscle, by Clarke and Cleghorn (2) to be deposited in considerable amounts in tissues remote from the area of trauma. The blood potassium levels are, however, not greatly increased under these circumstances except terminally (2, 15), but the function of vital tissues may be so depressed by the sudden acquisition of this and other toxic products of muscle damage that it is beyond the capacity of even a greatly increased cortical hormone secretion to maintain metabolic processes within normal limits. It is also possible that toxins from the damaged tissue may affect the adrenal gland itself deleteriously for changes that may be of sufficient severity to interfere with function are sometimes found in shocked animals (18) and in man following burns (8).

Toxins and relative or absolute deficiency of cortical hormones may be important as sustaining factors in shock once the process has been initiated by a decrease in blood volume. This would seem to be possible in experiments such as those of Solandt and Best (20) and of Freeman (4) in which local fluid loss at the site of trauma was insufficient to cause death by reduction in blood volume. Factors other than decrease in blood volume might also be suspected as important in those cases of shock in which apparently adequate restoration of the blood volume does not prevent death. This is seen both clinically and experimentally (5, 19) and shock is then said to have reached the irreversible stage. In order to study this phenomenon shock has been produced in dogs and an attempt has been made to identify the conditions and changes that determine success and failure attending therapy with blood substitutes.

Methods

Dogs of mixed breed and both sexes weighing 5 to 12 kg. were used. They were kept in the laboratory for several days so that those showing infection or losing weight could be discarded. Food, but not water, was withheld for at least 16 hr. before the production of shock by trauma and except where otherwise noted for 24 hr. after. The room temperature was 72° F.

Anaesthetic.—Pentothal sodium (Abbott Company), 20 to 30 mg. per kg. given intravenously as a 3% solution produced a surgical degree of anaesthesia lasting 15 to 20 min. A reinforcing dose of 3 to 5 mg. per kg. when the first signs of recovery appeared prolonged the period of unresponsiveness 10 to 15 min.

Trauma was administered during the period of surgical anaesthesia by many light blows to the thigh muscles with a light mallet whose striking surface was formed by a soft No. 6 rubber stopper. A soft stopper was essential since one with firm edges cut and caused considerable bleeding. This method of effecting muscle trauma has been widely used in the experimental production of shock (2, 13, 20, 22). Ninety-five blows per kilogram body weight was found to be approximately the suitable amount of trauma

in preliminary experiments. In the present experiments this amount was always given on one leg but, in animals showing an early fall of pressure towards 100 mm. Hg during the injury of the second leg, the number of blows was curtailed in order to prevent death occurring too soon after the trauma. Those showing little fall at the end of 95 blows per kg. on the second leg, however, received 5 to 15 more per kilogram on one or both legs depending on the level of the blood pressure. Administration of the trauma took about three minutes per kilogram. Neither skin nor bones were broken, nor was the muscle pulped. The animals surviving long enough to regain consciousness did not show manifestations of pain. Absence of complaint of pain is a clinical phenomenon in shock.

Arterial blood pressure readings were obtained from a carotid artery in which a simple semicurved cannula filled with a 0.1% heparin solution was inserted. The extruding end was connected with the manometer as desired; otherwise the free end was closed by a glass plug. The pressure in the manometer had to be adjusted to the expected blood pressure before taking a pressure reading. The cannula–manometer system is shown in Fig. 1. This cannula also provided an easy method of obtaining arterial blood samples. Slight infection of the neck wound was apparent in some animals surviving 24 hr. but appeared to be mild. In dogs living indefinitely the cannula generally sloughed out about the third day and the wound then healed.

Heart rate was followed by auscultation with a stethoscope.

Blood substitutes.—Pooled dogs' serum and a 4% solution of isinglass (22) were used. The amount generally given corresponded to approximately 45% of the blood volume estimated as 8.5% of the body weight. The transfusion was started when the blood pressure and heart rate indicated that death would take place in one-half to two hours without treatment.

Packed cell volume was estimated on heparinized arterial blood spun to a constant volume at 3000 r.p.m.

Blood sugar was determined, generally on arterial blood samples, by the method of Folin and Wu (9).

Results

It is not intended in this paper to discuss details of the experiments in which transfusions of serum or isinglass were given. This will be dealt with subsequently. It should be noted, however, that isinglass produced a rise in blood pressure as marked and sustained as serum produced and evinced no toxicity.

The estimated mortality of 195 dogs subjected to muscle trauma by the described technique is summarized in Table I.

One hundred and sixty-four dogs or 84% are considered as surviving less than 24 hr. Of these 119 received a blood substitute when death was considered certain within one-half to two hours. The estimated length of time that these animals would have survived without transfusion has been used

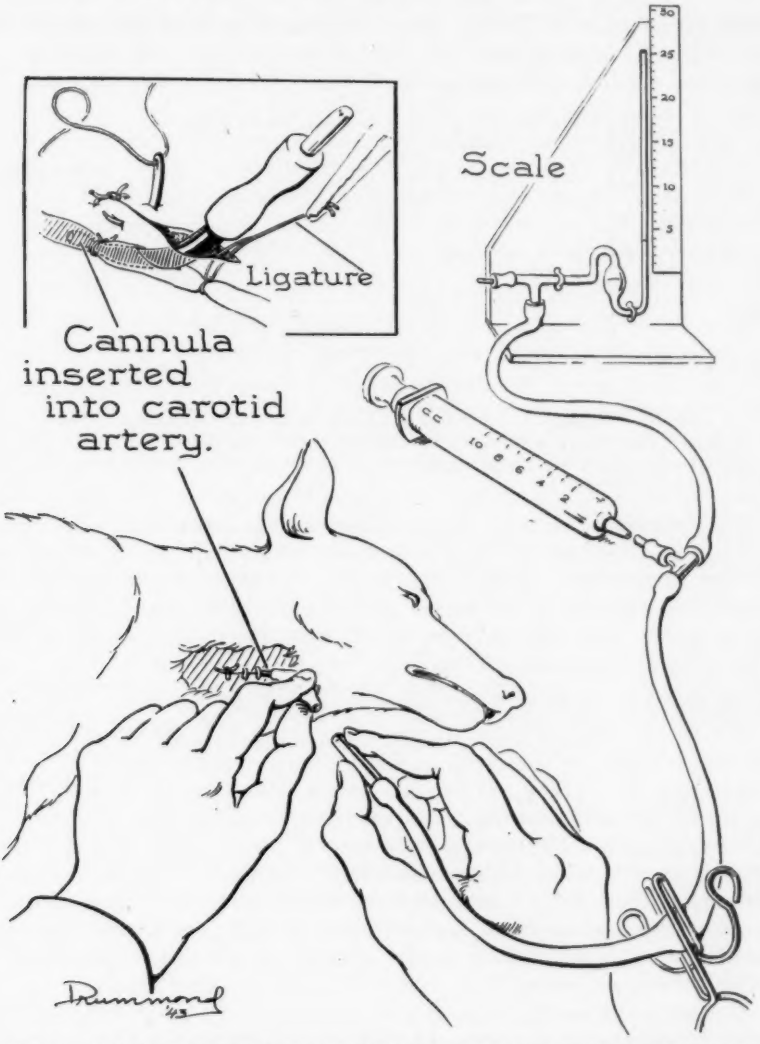

FIG. 1. *Method and apparatus used for obtaining repeated blood pressure readings in the conscious dog.*

TABLE I

ESTIMATED MORTALITY FOLLOWING MUSCLE TRAUMA

Classification	Number	Per cent
Survived or would have survived less than 24 hr. if untreated	164	84.0
Survived more than 24 hr., less than 80 hr. (untreated)	7	3.7
Survived indefinitely (untreated)	24	12.3

in the construction of Table I. Fig. 2 illustrates the incidence of fatal shock in the first 24 hr. after trauma. The greatest incidence fell in the three to three and one-half hour range, thereafter it fell off sharply. About half of

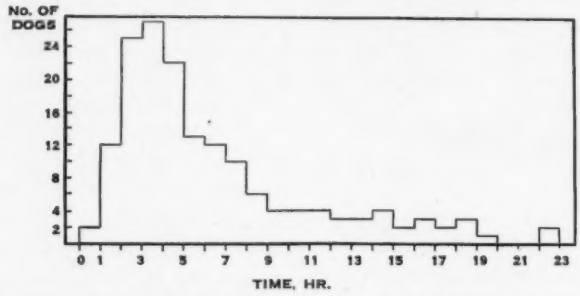

FIG. 2. *The incidence of fatal shock in relation to the time after trauma. The duration of survival of 45 dogs that died of shock and the probable duration of survival of 119 that received a blood substitute when in marked shock are plotted here. No effect of the treatment is in any way indicated.*

the dogs that developed what was apparently fatal shock if left untreated did so in the first five hours. Seven dogs, none of which were treated, died between 24 and 80 hr. after trauma. The 24 dogs called indefinite survivors were untreated. They appeared bright and were eating well at the end of three days, except three that were killed for examination at the end of 24 hr. when they appeared to be recovering.

Blood Pressure and Heart Rate Changes in Untreated Dogs

The blood pressure rose as trauma was started but soon began to decline. Trauma was usually discontinued when the blood pressure ranged from 90 to 100 mm.Hg. The blood pressure at the outset (initial pressure) varied considerably, making it difficult to compare the subsequent low pressure in different animals. For example, a pressure of 90 in an animal with an initial pressure of 180 was of far more serious import than in one with an initial pressure of 120 mm.Hg. In order to make the degree of hypotension after trauma comparable in different experiments the blood pressure was calculated as the per cent of the initial pressure, as in our previous experiments on shock following severe bleeding (3).

The heart rate at the start of trauma was usually greater than 180, an effect of the anaesthetic. By the time trauma was finished it usually had fallen considerably. A review of all fatal cases and survivors showed that if the blood pressure level was less than 65% of the initial at one and two hours after trauma and the heart rate more than 140, death was practically certain in less than five hours after trauma (Fig. 3, Nos. 1, 2, 3). A few dogs that lived longer and showed a blood pressure of less than 65% had heart rates at one and two hours of less than 130. Those rare cases that died in less than five hours despite a relatively high blood pressure at one and two hours had also a very high heart rate (Fig. 3, No. 4). In dogs living less than five hours

a rise to over 200 frequently occurred within one-half to one hour of death (Fig. 3, Nos. 2, 3, 5). In a few instances both blood pressure and heart rate were maintained at a relatively low level, consciousness returned yet death occurred, as a rule rather suddenly (Fig. 3, No. 6). In dogs surviving more than five hours there was usually an increase in heart rate within an hour of the end of trauma, associated with a rise in blood pressure (Fig. 3, Nos. 7, 8, 9, 10). The level of the blood pressure in these was usually between 65 and 85% of the initial pressure. A heart rate rising above 200 generally suggested that death would occur within a few hours. A decrease in heart rate generally indicated improvement.

Clinical Course in Untreated Dogs

The dogs dying in less than two hours rarely showed a return of motor activity. About half of those surviving two to five hours began to raise their heads in a co-ordinated fashion and tended to sit up one to two hours after trauma. They were, however, very apathetic and appeared much more sick than dogs destined to die in the same time as a result of simple haemorrhage. Blood-tinged and, sometimes, copious loose stools were passed by a few of these animals. Those that did not recover consciousness and co-ordinated activity often showed irregular bodily movements, at times walking movements and opisthotonus. When these phenomena were apparent the pressure in some of the animals was below 80, in others it was well over 100 mm.Hg. A few of these animals lived 12 to 24 hr. without ever showing a return of co-ordinated activity and consciousness. As a rule the dogs that survived longest showed the earliest and most complete return of consciousness and activity. With the approach of death apathy and unconsciousness returned and respiratory irregularities occasionally appeared.

Haemoconcentration

The initial packed cell volume represents the average of the value obtained just before, and that obtained 10 min. after anaesthetization. These values were averaged for the following reasons. In the unanaesthetized dog the proportion of cells is higher in the first than in later blood samples when the dog is quieter. In dogs anaesthetized with a barbiturate the packed cell volume decreases on account of the withdrawal of cells from active circulation (12). Consequently an average of values obtained before and during anaesthesia would come closer to the true resting value, the deviation of the changes being in opposite directions. Observations on 137 of the dogs that either died subsequently of shock or would have died had they not received treatment showed an average initial packed cell volume of $43.2 \pm 2.15\%$.

An increase in the volume of packed red blood cells, indicating haemoconcentration with respect to red blood cells, occurred in most cases. The maximal increase was usually attained two hours after trauma, as shown in a few experiments where a sufficient number of observations were made. Dilution was rarely observed except in dogs receiving transfusion with a blood substitute or in those surviving more than 24 hr. In the latter, dilution

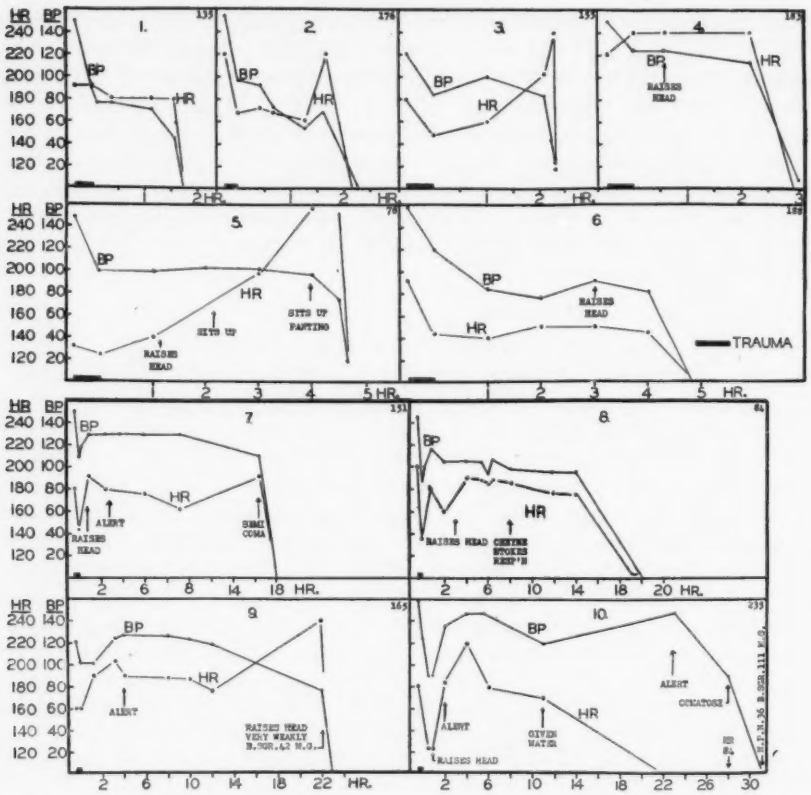

FIG. 3. *Blood pressure and heart rate changes after muscle trauma in untreated dogs.*

1. Dog 135.F; 8 kg.; 70 min. after trauma B.P. = 49% of initial pressure; H.R. 180; unconscious; dead in 1¾ hr. Autopsy—lungs normal, outside of bowel pale, mucosa of small bowel shows areas slightly congested; adrenal cortex lipoid filled, no congestion; legs show more blood than usual.

2. Dog 176.M; 6.4 kg.; 75 min. after trauma B.P. = 36% of initial pressure; unconscious; dead in 2¼ hr. Autopsy—outside of bowel pale; mucosa of duodenum and upper ileum moderately congested; adrenal cortex generally pale but shows small scattered areas of congestion; legs show considerable gross haemorrhage.

3. Dog 155.M; 10.3 kg.; 1 hr. after trauma B.P. = 83% of initial; H.R. 160; survival not predictable; 2¼ hr. B.P. = 72%; H.R. 200; death certain soon with rising H.R. and falling B.P.; unconscious throughout; dead in 2¾ hr. Autopsy—no apparent congestion of any viscera; adrenal cortex lipoid filled.

4. Dog 183.F; 10.6 kg.; 40 min. after trauma B.P. = 83% of initial; H.R. 240; raises head; 2¼ hr. B.P. = 76%; H.R. 240; long survival improbable but no certain indication early approach of death; dead in 3 hr. Autopsy—moderate congestion of mucosa of duodenum and colon; adrenal medulla shows haemorrhage that involves inner margin of cortex as well.

5. Dog 78.M; 10.9 kg.; 1 hr. after trauma B.P. = 66% of initial; H.R. 140; raises head; 2 hr. B.P. = 67%; sits up; 3 hr. B.P. = 67%; H.R. 200; outlook poor; 4 hr. B.P. = 63%; H.R. 260; panting; moribund; dead 4¾ hr. Autopsy—lungs appear slightly congested; mucosa of stomach and small bowel slightly congested; mucosa of colon intensely congested; kidneys show congestion on section; adrenals not examined.

began to be evident some six to eight hours after trauma although water was generally not given till 24 hr. after trauma.

The average packed cell volume at the time of the maximal reading in the 137 dogs was 51.2 ± 2.47%. In six of these no increase occurred. In 15 more the increase was slight, being 10% or less. The maximal increase observed was 62% above the initial value. Fig. 4 illustrates the degree and frequency of the increase in the packed cell volume in these dogs expressed as a per cent of the initial value.

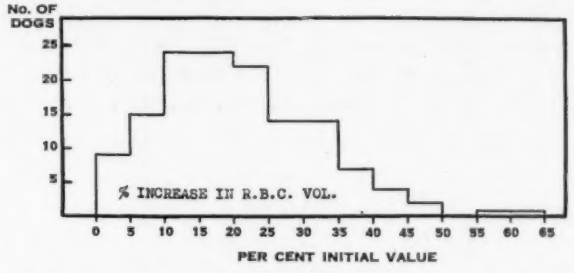

FIG. 4. *The percentage increase in the red blood cell volume of 137 dogs after muscle trauma.*

The degree of dilution effected by administration of the non-cell-containing blood substitutes was recorded. The average packed cell volume in 95 cases,

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6. Dog 188.M; 11 kg.; 1 hr. after trauma B.P. = 53% of initial; H.R. 140; 2 hr. B.P. = 51%; H.R. 150; 3 hr. B.P. = 56%; H.R. 150; raises head; relatively very low pressure; might recover if heart rate picked up; 4 hr. B.P. = 50%; H.R. 144; outlook poor with B.P. falling to lower level; dead in 4¾ hr. Autopsy—duodenal mucosa shows severe congestion and bloody fluid in lumen; mucosa of colon severely congested; adrenals, diffuse moderate congestion of cortex; considerable blood has tracked up groins.

7. Dog 151.M; 9.9 kg.; 75 min. after trauma B.P. = 86% of initial; H.R. 192; holds head up; 6 hr. B.P. same; H.R. 174; recovering ?; 16¼ hr. B.P. = 75%; H.R. 192; semicomatose; respirations 32, forced; blood sugar 42 mg. %; dead in 18¾ hr. Autopsy—lungs slightly congested; mild to moderate congestion of mucosa of large and small bowel; adrenal swollen; medulla haemorrhagic; cortex very congested. Illustrated.

8. Dog 84.M; 8.7 kg.; 75 min. after trauma B.P. = 79% of initial; H.R. 180; 2 hr. B.P. = 70%; H.R. 162; raises head; 4 hr. B.P. = 70%; H.R. 188; 6 hr., quieter; loose stool; 8 hr. B.P. = 66%; H.R. 180; Cheyne Stokes' breathing; semicomatose; 14 hr. B.P. = 64%; H.R. 176; respiration still irregular; died between 18 and 21 hr. after trauma. No autopsy.

9. Dog 165.F; 8.6 kg.; 1 hr. after trauma, B.P. = 85% of initial; H.R. 192; 3 hr. B.P. = 104%; H.R. 204; awake, alert; 12 hr. B.P. = 100% initial; H.R. 176; raises head, wags tail, appears to be recovering; blood sugar 63 mg.%; 22 hr. B.P. = 65%; H.R. 240; can just raise head weakly, blood sugar 42 mg.%; 22¾ hr. dead; blood sugar 28 mg.%. Autopsy—mild to moderate congestion of duodenum and colon; adrenals, severe cortical congestion and haemorrhage. Illustrated.

10. Dog 235.M; 11.2 kg.; 75 min. after trauma, B.P. = 60% of initial; H.R. 128; raises head; 2 hr. after trauma, B.P. = 85%; H.R. 156; 3 hr., B.P. = 91%; H.R. 220; 11 hr., B.P. = 77%; H.R. 174; alert, appears to be recovering; given water, took 1500 cc.; 22½ hr., B.P. = 91%; H.R. 114, not so alert; has passed only 195 cc. dark urine; takes 750 cc. water; 28 hr., comatose, B.P. = 62%; H.R. 84; obviously dying; N.P.N. 36 mg.%; blood sugar 111 mg.%; 30¼ hr. dead. Autopsy—lungs slight congestion only; very slight, patchy congestion of mucosa of small bowel; kidneys and liver appear to be congested on section; adrenals—severe confluent haemorrhage and congestion throughout cortex, most intense at junction of inner and middle third; heart blood culture negative.

after the transfusion, was 32.1 ± 2.12%, the average amount of blood substitute given being about 45% of the blood volume calculated as 8.5% of the animal's body weight. In 17 untreated dogs surviving indefinitely the average initial packed cell volume was 42.1 ± 2.12%. The average value at the time of the maximal increase was 45.2 ± 2.56%. Five of the 17 showed a percentage volume increase of less than 10%. In the remaining 10 the average increase was 19%. The blood volume reduction was obviously less in this group.

Blood Sugar

Observations were made on some 60 dogs. In Fig. 5 the values obtained on 42 dogs at or near death are charted. The blood sugar values are plotted against the time after trauma at which the blood sample was obtained. This

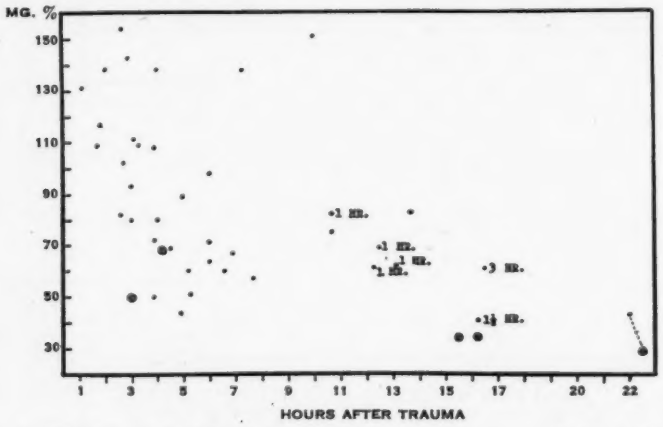

FIG. 5. *Blood sugar level in 42 dogs dying after muscle trauma. Points without other marking indicate values obtained within one-half hour of death. Points marked with hours adjacent indicate time before death when value was obtained. Points circled indicate values obtained at the time of death.*

was generally about one-half hour before death actually occurred or before death would have occurred had not treatment been instituted. In certain cases indicated, the blood samples were obtained one to three hours before death.

In 18 dogs that died, or would have died within four hours of trauma, the samples that were obtained shortly before death was anticipated showed values less than 80 mg. % in three cases only. Four were above 130 mg. %. In animals dying more than four hours after trauma the trend was towards lower values and in three samples after the 15 hr. period values below 35 mg. % were obtained at death. The decrease in blood sugar occurred more rapidly with the approach of death.

Moderate prolongation of life by transfusion with a blood substitute was accompanied in a majority of experiments by a great decrease in blood sugar.

The dilution of the blood by the blood substitute cannot account for the fall in blood sugar after the infusion. In Fig. 6 are shown the blood sugar levels in 13 dogs before and after treatment with a blood substitute. The first

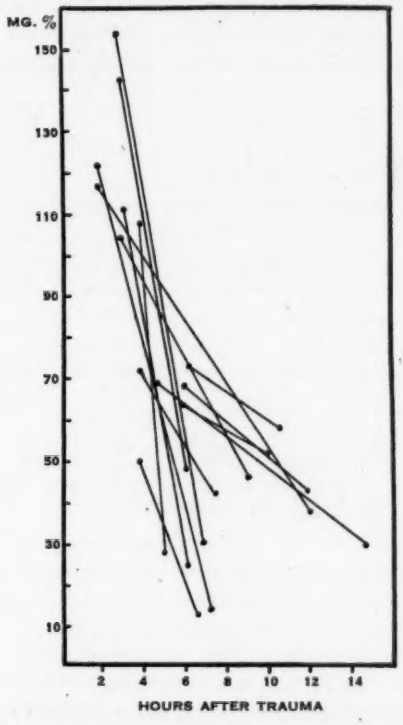

FIG. 6. *Effect of moderate prolongation of life by blood substitute on blood sugar of dogs dying after muscle trauma. First point, value obtained within one hour of expected death when blood substitute started. Second point on each curve represents value obtained within one-half hour of death.*

value was obtained on blood secured about one-half hour before death was expected and just before treatment was instituted. In only one of these was the value less than 63 mg. %. Then the second value was obtained on blood secured about one-half hour before death actually occurred, life having been prolonged 2 to 15 hr. In only two cases was the final value more than 50 mg. %. Intermediate values between the first and final were obtained in many experiments but did not show significant variation. Glucose was injected intravenously in six dogs just after the completion of the blood substitute transfusion. As a result of the 12 gm. of glucose given, blood sugar values rose greatly, as high as 350 mg. %, but two dogs died within an hour despite high blood sugar levels. Three lived from two and one-half to five hours after the glucose infusion. In all these the blood sugar had declined

to values below 50 mg. % in three hours or less, death occurring an hour or two after. One lived more than 24 hr. or beyond the period influenced by the glucose infusion.

A more marked prolongation of life, as shown in Fig. 7, was rarely associated with so precipitous and never with so severe a fall in blood sugar. All these dogs survived more than 15 hr. A serious fall in blood pressure in many

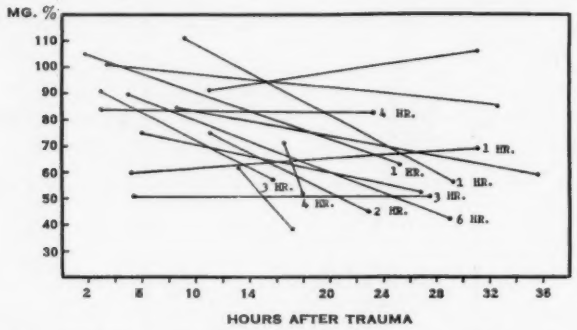

FIG. 7. *Effect of prolongation of life beyond 15 hr., by a blood substitute, on the blood sugar of dogs dying after muscle trauma. First point, value obtained within two hours of expected death when blood substitute was started. Second point on each curve represents value obtained within one-half hour of death or at number of hours before death indicated adjacent to the point.*

dogs in this group did not develop for many hours after trauma. The first blood sugar value charted was obtained about one-half to two hours before death was expected, i.e., just before treatment was instituted. In only one case was this value less than 69 mg. %. The final value was less than 40 mg. % in one case only, but in five others the blood was secured one or more hours before death. It seems likely that in some of these cases the value at a point nearer death might well have been below 40 mg. %. What is more important is that in four cases the value within one-half hour of death was above 52 mg. %, and in three others it was above 56 mg. % one hour before death. These were all animals surviving more than 24 hr. Serum did not appear to be superior to isinglass either in preventing death or fall in blood sugar.

Discussion

The technique of applying muscle trauma was designed to obtain as many cases of delayed shock as possible. There were, however, more dogs developing severe shock earlier than five hours than later. Those that died in two or three hours rarely showed a return of consciousness. It is not believed that this was solely due to the persistence of the anaesthetic for dogs may have pressures just as low as many of those caused by haemorrhage following pentothal treatment yet show a return of consciousness (3). Furthermore, dogs dying later, after a period of consciousness, became apathetic and lost

consciousness at blood pressure levels well above those consistent with consciousness and co-ordinated activity following bleeding.

The fact that some untreated dogs died between 24 and 80 hr. is important. It indicated that in any attempt at therapy the criterion of success obviously had to be the extension of survival beyond the period during which death could be attributed to the effects of the injury inflicted. This criterion has seldom been adhered to in work on shock.

Frequent observations of the blood pressure alone following trauma gave a fair indication of the approach of death in some animals, particularly in those dying within five hours. In some of these, however, and in those that survived longer, the blood pressure alone had little or no prognostic significance. In some dogs the blood pressure was relatively well maintained, but suddenly fell and within 15 to 30 min. the animal died. Observations on changes in heart rate, when considered in conjunction with blood pressure changes after trauma, made it possible to predict the approach of death with some certainty.

Haemoconcentration is not necessarily a sign of shock. Its occurrence depends on two factors. First, it develops in cases where the blood volume reduction is principally plasma, rather than whole blood. Secondly, haemoconcentration is more apt to be observed in animals with vasomotor responses intact as in our experiments in which pentothal sodium was used, than in animals anaesthetized with a barbiturate (such as nembutal) that has a longer effect and contributes to splenic relaxation with consequent withdrawal of cells from the circulation (12). In this connection it is important to note that Taylor and Page (23) have shown that 65% of the increase in packed cell volume in dogs in tourniquet shock is due to the discharge of cells from the spleen. The type of damage produced by multiple light blows to the thigh muscles, though requiring but about 30 min. to produce in a 10 kg. dog, is somewhat similar to that elicited in dogs subjected to occlusion of the circulation in the hind limbs for four or five hours. There is, however, more red cell loss in the former condition than in the latter. Haemoconcentration was infrequently observed by Manery and Solandt (15), in dogs under nembutal following muscle trauma. In certain of our animals it was not apparent, yet the animals died. In such cases evidence of haemorrhage was more marked in the traumatized muscles than in those in which haemoconcentration was present.

Our findings show that normal or high blood sugar values are usually found within one-half hour of death in animals dying within four hours of trauma. Hyperglycaemia is traditionally associated with shock, but is probably dependent on observations made on cases in early rather than the late stages. The cause of the hyperglycaemia is the discharge of adrenalin, for, as Engel, Winton, and Long (7) have shown, it does not occur in rats in which the adrenal medulla has been previously removed. Many very low values were obtained in dogs dying later than four hours. Terminal hypoglycaemia was observed in shocked rats by Engel *et al.* (7) and by Govier and Greer (11) in dogs. The hypoglycaemia, and other metabolic changes in shock have been

attributed to early anoxia of the liver and an increased glucose utilization by peripheral tissues by Engel, Winton, and Long. Evidence of liver damage, to be reported in detail subsequently, was observed in our dogs in liver sections. A similar though less marked change has been described by other workers, Freeman *et al.* (10), Dunphy, Gibson, and Keeley (6), and others. It is of interest that it was particularly marked in an animal in which life had been moderately prolonged by a blood substitute and that it was in such animals that the most marked and rapid fall in blood glucose occurred. Presumably the liver changes were so severe that the improved circulatory conditions following restoration of the blood volume were not enough to restore liver function. The experiments in which the blood sugar fell rapidly after hyperglycaemia induced by infusion of glucose may be interpreted as due in part at least to the increased rate of utilization of glucose by the peripheral tissues (7). Blood sugar values were within normal limits in many dogs whose lives were prolonged beyond 24 hr. by a blood substitute and as will be shown elsewhere they did not show marked liver damage. The cause of death in these animals was probably not the same as in those dying earlier with hypoglycaemic sugar levels and as will be shown in a later paper such deaths may be ascribed in part at least to the severe renal and adrenal changes found.

Elimination or severe suppression of the activity of vasomotor reflexes by anaesthesia cannot but hinder the development or obscure the recognition of certain phenomena that occur in shock. Prolonged anaesthesia by ether or pentobarbital sodium can lead to changes in splanchnic viscera similar to those seen in shock (1). The use of the evanescent barbiturate pentothal sodium in our experiments resulted in a reduction of these hazards to a minimum.

Acknowledgments

The authors are indebted to Prof. Duncan Graham for his support and criticism, and to Dr. C. N. H. Long for suggesting the importance of blood sugar studies. Miss M. I. Hanna kindly made the blood sugar estimations. The separation of the serum was done through the kindness of Dr. A. M. Fisher and the isinglass supplied by Dr. N. B. Taylor. Mr. Walter Cowan rendered expert technical assistance which is gratefully acknowledged.

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THE CARDIAC ACTION OF POSTERIOR PITUITARY EXTRACT IN PHYSIOLOGICAL DOSES, IN THE NORMAL DOG, AND AFTER PARTIAL AND COMPLETE DENERVATION OF THE HEART[1]

By Margaret E. MacK. Sawyer[2] and G. H. Ettinger[3]

Abstract

In the normal dog continuous infusion of dilute posterior pituitary extract produces a maximal inhibition of the heart, i.e. slowing to about one-half of the resting rate, with usually a rise in blood pressure of 10 to 30 mm. of mercury.

After bilateral thoracic sympathectomy, posterior pituitary extract also produces maximal inhibition. This inhibition, like that produced in the normal dog, is abolished by atropine.

After bilateral vagotomy posterior pituitary extract produces a moderate but not maximal inhibition. This inhibition is not abolished by atropine.

After bilateral thoracic sympathectomy and unilateral vagotomy, posterior pituitary extract produces a maximal effect.

After total denervation of the heart, posterior pituitary extract produces no inhibition of the heart and the rate is unchanged.

Characteristic changes are produced by posterior pituitary extract in the electrocardiogram of normal dogs. After total denervation no change takes place.

It is concluded that the slowing of the dog's heart that is produced by continuous intravenous infusion of posterior pituitary extract is entirely due to its action through the inhibitory fibres of the vagus and sympathetic nerves.

The bradycardia produced in the dog by posterior pituitary extract, according to Resnik and Geiling (11), and Gruber and Kountz (4), is due to a two-fold action: (*a*) a reflex vagal slowing, and (*b*) a direct depressant action on the myocardium, possibly through coronary arterial constriction. Melville (7) considers that there is no direct myocardial depression; all changes apart from vagal slowing are caused by constriction of the coronary arteries. In the cat, Bacq and Dworkin (1) obtained results that indicated that the slowing produced by pitressin was entirely due to an action upon the extra-cardiac connections of the cardiac nerves.

In order to re-explore the site and nature of the inhibitory process, we determined the action of posterior pituitary extract in the normal dog, and in the dog after partial and complete cardiac denervation. These experiments were done in the course of an investigation of the effects of chronic excitation of the inhibitory mechanism of the dog's heart, by infusion of a dilute solution of posterior pituitary extract for two hours daily, over a period of four and one-half years.

Previous investigators tested the action of posterior pituitary extracts, including pitressin, by injecting large single doses of commercial or other

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[2] *Fellow in Physiology.*

[3] *Professor of Physiology.*

concentrated preparations; these doses are more liable to be toxic than physiological. The results were often complicated by the use of anaesthesia. In our experiments a more nearly physiological method of administration was used. Posterior pituitary extract was slowly administered in dilute solution by continuous infusion for 30 to 120 min. to trained, unanaesthetized dogs weighing from 12 to 19 kg., at such a rate as to produce a continuous maximal bradycardia without disturbing effects such as nausea or vomiting. This required only about two to two and one-half pressor units per hour.

Method

Dogs were trained to lie quietly on a table, until their resting-heart rates could be obtained accurately before each infusion was started. After application of alcohol to the leg, a sterile hypodermic needle was inserted into a leg vein and taped in place. This was connected to an outlet on the bottom of an elevated sterile flask containing the infusate by a sterile rubber tube, on the course of which was an air trap by which the rate of flow could be determined in drops. Infusion by gravity flow was continued for 30 to 120 min. and the heart rate counted every 5 to 10 min. Occasionally a continuous count was made for five minutes after the start of the infusion to obtain any immediate changes. After a few trials the dogs remained quiet throughout the infusion, which usually seemed to have a sedative effect.

The pituitary extract used was prepared by the Connaught Laboratories, Toronto, Ont. It contained 10 pressor units per cc., and had tricresol, 0.1% as a preservative. A solution was made of 1 cc. of the extract dissolved in 500 cc. of sterile normal saline. Each animal received as much as 250 cc. of this dilute solution. Daily infusion for periods up to four and one-half years produced little evidence of a tolerance (see Table I).

TABLE I

THE EFFECT OF INFUSION OF POSTERIOR PITUITARY EXTRACT ON THE HEART RATE OF THE DOG, NORMAL, AND WITH CARDIAC SYMPATHECTOMY, REPEATED DAILY OVER A PERIOD OF YEARS

Dog	Condition	Resting rate per minute	Minimal infusion rate, per min.	Resting rate per minute	Minimal infusion rate per min.
		1938		1942	
Sandy	Normal	64	30	60	44
Toy	Normal	66	40	80	44
Scarface	Normal	68	30	76	36
Rufus	Normal	72	40	88	44
		1940		1942	
General	Cardiac sympathectomy	64	36	72	44
Sargent	Cardiac sympathectomy	68	44	68	40
Ted	Cardiac sympathectomy	72	40	76	44

The effect of atropine (0.2 to 0.3 mg./kg.), administered intravenously before infusion and during infusion, was determined in all dogs after repeated experiments had demonstrated the normal response to posterior pituitary extract.

The effect of the infusion on blood pressure was determined by connecting a mercury manometer to a sterile needle or arterial cannula inserted into the femoral artery under local anaesthesia.

Cardiac denervation was done after the normal response to posterior pituitary infusion had been recorded. The response to infusion and the effect of atropine on the response were determined after operative recovery from each step in the denervation. The sympathetic nerve supply to the heart was interrupted by aseptically removing the stellate ganglia and the thoracic sympathetic chains down to the ninth rib, in two steps, after the method of Cannon *et al.* (2). Cervical vagotomy was done in two stages by aseptic removal of 3 cm. of each of the nerves. At least six days were allowed to elapse between operations. In most cases a period of several weeks passed. Complete denervation of the heart was done in three stages: first, right thoracic sympathectomy and right cervical vagotomy; second, left thoracic sympathectomy; third, left cervical vagotomy. Excision of the vocal cords at the time of the first stage prevented any later difficulty in respiration. Completely vagotomized dogs retained little food, but were maintained for periods of 10 days to two months by supplementing feeding with intravenous infusion of glucose saline.

Electrocardiograms were taken on all animals at all stages of the experiment.

Results

A. Normal Animals

The Effect of Posterior Pituitary Infusion on the Heart Rate

The resting-heart rates in the trained dogs were from 60 to 90 per minute. Continuous infusion with posterior pituitary extract invariably inhibited the heart to about half the resting rate (Table I). This inhibition commenced about one to two minutes after the start of infusion, became maximal in 5 to 10 min. and was maintained thereafter as long as the infusion continued. There was sometimes a very brief period of acceleration in the first minute immediately after the start of infusion, before the heart began to slow. (This acceleration was always observed by Gruber and Kountz (4) and by Resnik and Geiling (11) who used large single injections of extract.) After the infusion was stopped the heart rate gradually increased and came back to the resting rate in 20 to 30 min. Figs. 1 and 2 show characteristic changes in heart rates in normal dogs during infusion with posterior pituitary extract.

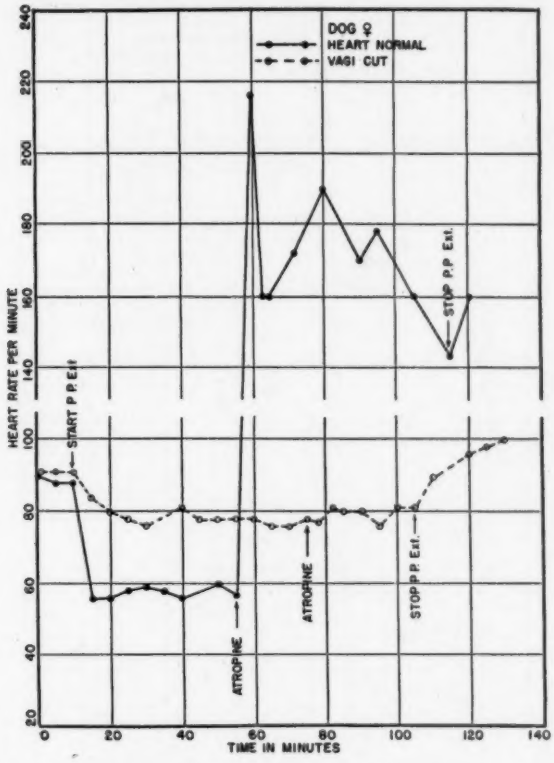

FIG. 1. *The effect of posterior pituitary infusion and the subsequent injection of atropine on the heart rate of a normal, unanaesthetized dog before and after section of the vagi.*

The Effect of Atropine on the Response to Posterior Pituitary Infusion

The effect of atropine varied according to whether it was administered before the infusion had started, or during the infusion. Given without infusion it produced a tachycardia up to 200/min. within five minutes, followed by a slow and steady decline in rate, with a return to the normal in one and one-half to two hours. When pituitary extract infusion was started 10 to 30 min. after the atropine injection, it usually produced no inhibition. The heart either maintained the atropine effect with its gradually declining influence, or there was a brief period of further acceleration (Fig. 3). This latter response is similar to that reported by Resnik and Geiling (11) following a single injection of posterior pituitary extract. Gruber and Kountz (4) found that pitressin slightly slowed the atropinized heart.

When atropine was administered during infusion with posterior pituitary extract, and while the bradycardia was maximal, it invariably produced an intense tachycardia within two minutes (Figs. 1 and 2). The heart rate was

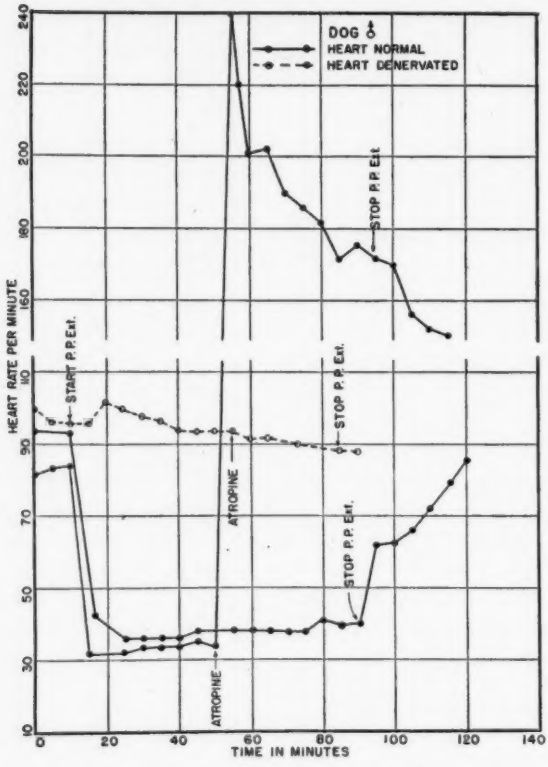

FIG. 2. *The effect of posterior pituitary infusion and the subsequent injection of atropine on the heart rate of a normal, unanaesthetized dog before and after complete denervation of the heart.*

higher than that producible in the same dog by atropine alone. For example, in a dog in which infusion of posterior pituitary extract normally slowed the heart rate from 62 to 34 per minute, and in which atropine without infusion raised the heart rate to 210 per minute, atropine during infusion raised the rate to 244 per minute.

It is clear that atropine prevents the bradycardia produced by posterior pituitary infusion.

Electrocardiographic Changes Produced by Posterior Pituitary Infusion

A number of investigators have studied the effect of single injections of posterior pituitary extract and pitressin on the electrocardiogram. A list of references may be found in a paper by Melville (7). Electrocardiograms taken during continuous infusion showed many changes similar to those reported after single injections (Figs. 5 and 6). Within two minutes of the start of infusion a marked slowing in rate took place, sometimes accompanied by an

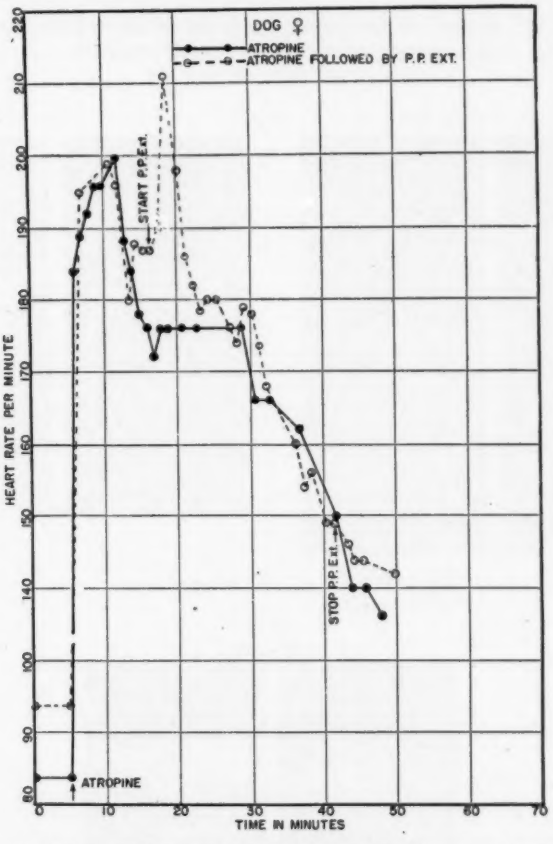

FIG. 3. *The effect of atropine, and atropine followed by infusion of posterior pituitary extract, on the heart rate of a normal, unanaesthetized dog.*

increased *P–R* interval. Changes in the *T* wave were most frequently observed. Often within 30 sec. of the start of the infusion it became much more prominent, its amplitude increased and its shape peaked. A *T* wave inverted or diphasic before infusion might become upright and more prominent during infusion. Changes in the *P* wave were not common. Occasionally it became slightly notched. Sometimes heart block occurred. Grouping of beats was occasionally seen after infusion had gone on for some time; *pulsus bigeminus* was the most common of these.

The tachycardia produced in the normal dog by atropine was accompanied by a shortened *P–R* interval, without disturbance of the *T* wave. Infusion of posterior pituitary extract after atropine produced, within one minute, the characteristic heightening of the *T* wave, but no other changes. Atropine injected during the infusion produced a lengthened *P–R* interval, heart block,

EFFECT OF CONTINUOUS INFUSION OF POSTERIOR PITUITARY EXTRACT ON THE BLOOD PRESSURE AND HEART RATE OF THE DOG

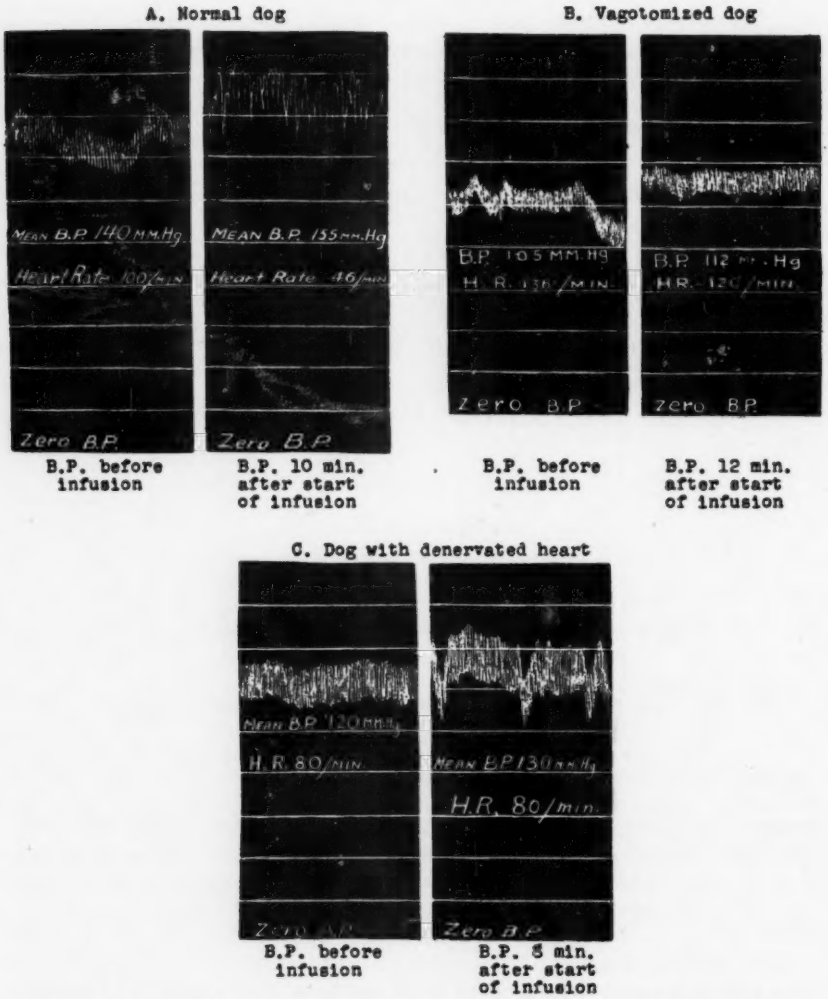

FIG. 4. *Change in femoral arterial blood pressure produced by infusion of posterior pituitary extract under local anaesthesia in the dog, normal, after vagotomy, and after complete denervation of the heart.*

irregularity with *pulsus bigeminus* and *trigeminus*, all within the first half-minute. The *T* wave remained high. After this brief irregularity a marked regular acceleration occurred, with shortened *P–R* interval, the *P* and *T*

waves frequently superimposed (Figs. 5 and 6). Gruber and Kountz (4) noted similar effects of atropine given two to three minutes after a single large dose of pitressin.

The Effect of Posterior Pituitary Infusion on Blood Pressure

A single large dose of pitressin or pituitary extract in the unanaesthetized dog produces (3, 4, 6, 7, 14) a sharp fall in blood pressure, preceded by a slight rise and followed by a second rise. A smaller dose may produce a pure pressor effect. Anaesthetics, particularly chloretone, intensify the pressor action (3, 10). Coronary dilators such as ephedrine and adrenaline also increase the pressor effect of small doses and may nullify the depressor effect of large doses (8).

The effect of infusion of posterior pituitary extract on blood pressure was registered in seven normal unanaestheized dogs. (For examples, see Table II, Fig. 4A). A pressor effect of 10 to 30 mm. of mercury was obtained within

TABLE II

CHANGES IN HEART RATE AND BLOOD PRESSURE PRODUCED IN THE DOG UNDER LOCAL ANAESTHESIA BY INFUSION WITH POSTERIOR PITUITARY EXTRACT

Dog	Condition	*Resting heart rate per min.	Minimal infusion heart rate per min.	Fall in* heart rate per min.	Resting blood pressure, mm. Hg	Infusion blood pressure, mm. Hg	Change, mm. Hg
Barney	Normal	100	46	54	140	165	+25
Gunner	Normal	90	64	26	140	150	+10
Major	Normal	114	80	34	170	180	+10
Mike	Normal	72	52	20	130	125	− 5
General	Cardiac sympathectomy	120	76	44	140	160	+20
Ted	Cardiac sympathectomy	88	60	28	140	160	+20
Sargent	Cardiac sympathectomy	100	76	24	140	170	+30
Sally	Vagotomized	136	105	31	105	112	+ 7
Sambo	Vagotomized	148	132	16	110	120	+10
Katy	Vagotomized	120	110	10	115	135	+20
Colonel	Total cardiac denervation	80	80	0	120	135	+15

* *The slight restlessness of the animals under the conditions of the experiment raised the resting rate and prevented maximal inhibition.*

30 sec. in six unanaesthetized dogs, and a fall of 5 mm. in one. The maximal cardio-inhibition followed the maximal pressure rise, but the heart was slowed even in the animal that showed the depressor effect. The pressure slowly fell with continued infusion, sometimes reaching the pre-infusion level. The bradycardia was maintained, however, and the rate did not return to the

resting level until 20 min. after cessation of infusion. These results indicate that the vagal effect does not depend on a depressor reflex.

B. Dogs with Thoracic Sympathectomy

Thoracic sympthectomy, with removal of stellate ganglia, was done in four dogs. This did not alter the resting-heart rate of 60 to 90 per minute. Two weeks after the final operation, posterior pituitary infusions were given. This produced an inhibition indistinguishable from that in the normal animal (Table I), with normal blood pressure elevation (Table II) and electrocardiographic effects, and with normal recovery time. Bacq and Dworkin (1) found that single injections of pitressin in the completely sympathectomized cat produced the same inhibition as in the normal cat, although the recovery time was prolonged.

Atropine injected during the bradycardia produced by infusion caused the typical tachycardia seen in the normal dog.

C. Vagotomized Dogs

Samaan (13) and others found that section of both cervical vagus nerves in the anaesthetized dog is followed by extreme cardiac acceleration. A similar increase in basal heart rate has been reported for the unanaesthetized cat (1, 9).

In this investigation bilateral vagotomy was done in three dogs whose response to posterior pituitary infusion, with and without artopine, had been established. Two of these had higher resting-heart rates than had most of the experimental animals. No change in heart rate occurred after section of one vagus. After double vagotomy one animal showed no change in basal heart rate (Fig. 1), the second showed a slight rise, and the third, a considerable acceleration (Table III). This third animal had respiratory irregularities probably due to inability to accommodate in the short interval (six days) between section of the two nerves.

The Effect of Posterior Pituitary Infusion on the Heart Rate

After double vagotomy the inhibition produced by posterior pituitary infusion was considerably less than that which occurred in the normal animal (Table III). Its onset was delayed and did not reach a maximum until 20 min. after the start of infusion (Fig. 1). It was then maintained but gradually ceased after withdrawal. Blood pressure changes during infusion were similar to those obtained in the normal animal (Table II, Fig. 4B). Infusion produced the following electrocardiographic changes (Fig. 5): lengthening of the P–R interval, inversion of T wave, increase in T voltage. There was no grouping of beats. The inversion of the P wave reported by Melville (7) was not seen.

The Effect of Atropine on Posterior Pituitary Inhibition

Atropine has been reported to cause cardiac acceleration after double vagotomy in the cat (9) and the dog (12).

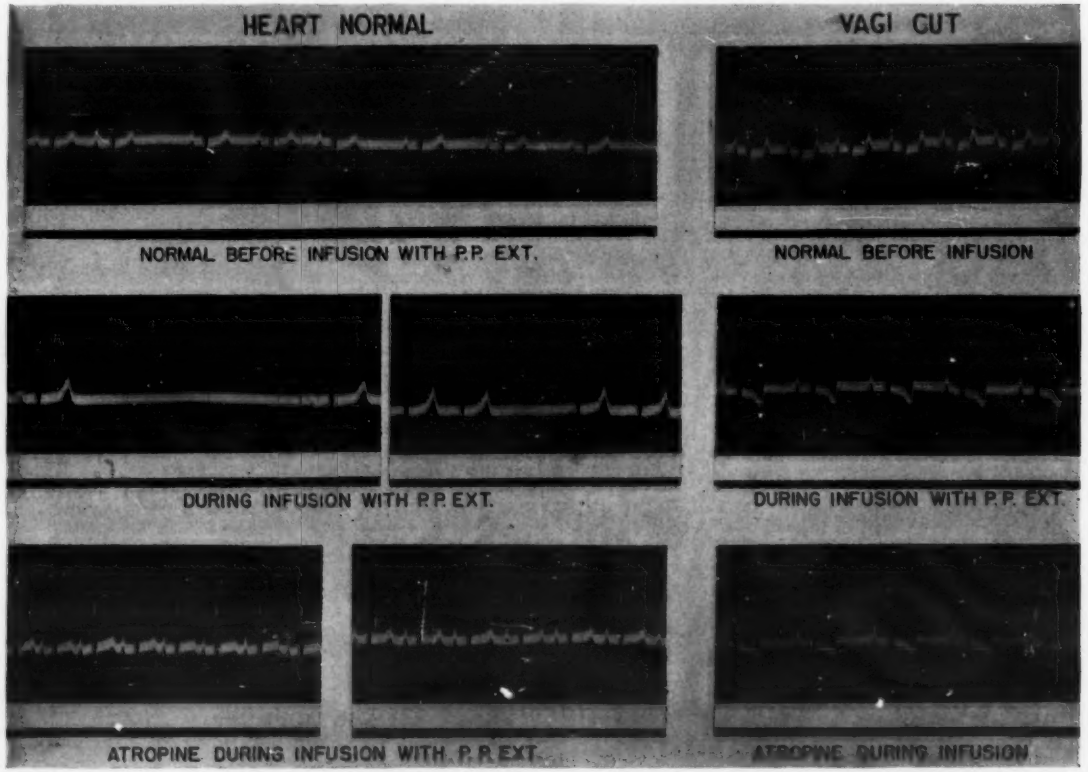

FIG. 5. *The effect of posterior pituitary infusion and the subsequent injection of atropine on the electrocardiogram of a dog before and after section of the vagi (Lead II).*

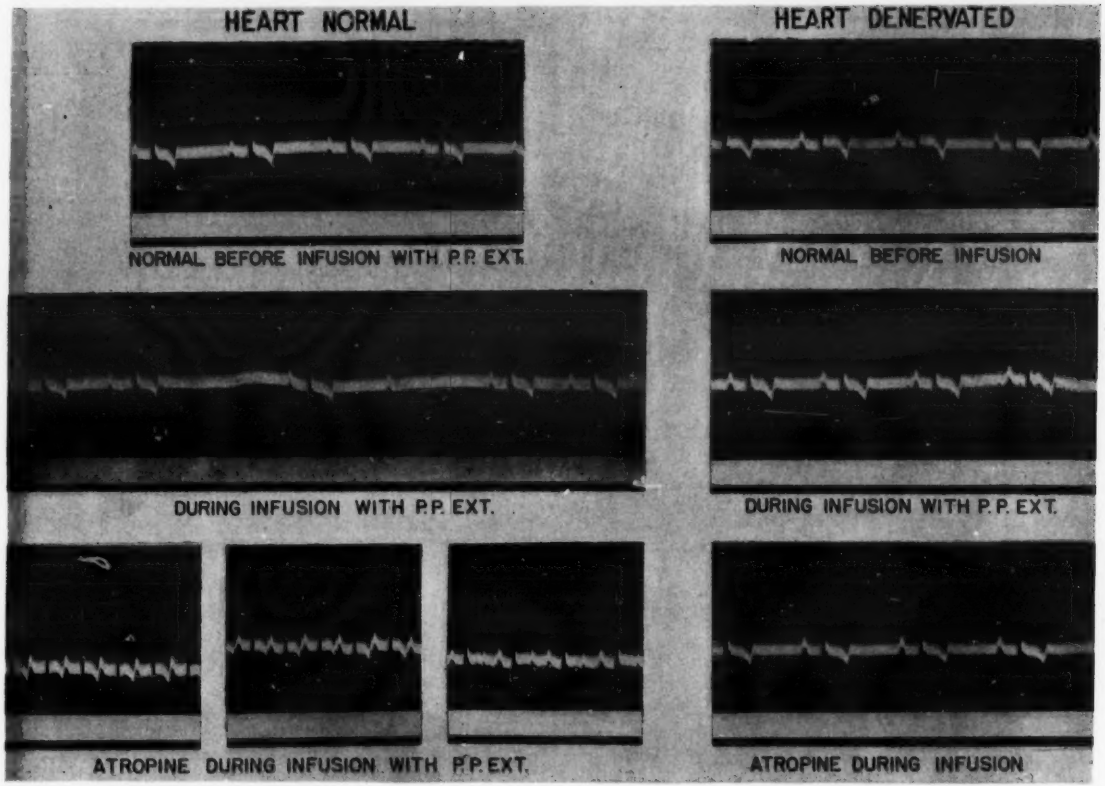

FIG. 6. *The effect of posterior pituitary infusion and the subsequent injection of atropine on the electrocardiogram (Lead II) of a dog before and after denervation of the heart. These records are from the animal that showed the maximal increase in basal heart rate after vagotomy.*

TABLE III

THE EFFECT OF POSTERIOR PITUITARY EXTRACT ON HEART RATE BEFORE AND AFTER VAGOTOMY

Dog Sally			Dog Katy			Dog Sambo		
Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate	Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate	Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate
Before vagotomy			Before vagotomy			Before vagotomy		
102	48		110	60		90	40	
100	56		90	43		74	36	
84	56		124	56		84	44	
102	48		96	60		66	34	
88	56	42			51	74	37	39
After unilateral vagotomy			After unilateral vagotomy					
102	83		102	54				
96	68		102	56				
90	68	23			47			
After double vagotomy			After double vagotomy			After double vagotomy		
104	92		132	108		138	122	
100	86		124	104	27	148	128	18
90	76							
96	80							
98	80							
134	118	15						

We were not able to demonstrate this at any time in three doubly vagotomized dogs, either immediately after section or two weeks later. When atropine was given while the heart was inhibited by pituitary infusion, there was no acceleration (Fig. 1). Given before infusion, atropine did not interfere with the inhibitory action of posterior pituitary extract. Atropine did not alter the electrocardiogram of the vagotomized dog under influence of posterior pituitary extract (Fig. 5).

It is significant that in the dog with a sympathetic cardiac denervation only, posterior pituitary infusion still exercises an inhibition on the heart, even in the presence of atropine.

D. Dog after Unilateral Vagotomy and Bilateral Thoracic Sympathectomy

Several experiments were done in one animal after recovery from single right vagotomy and double thoracic sympthectomy.

Infusion with posterior pituitary extract produced a slowing of the heart equivalent to that produced when the same heart had its normal innervation.

Atropine caused the same acceleration as was produced before the partial denervation, and overcame the bradycardia produced by posterior pituitary infusion just as in the normal dog.

E. Animals with Complete Denervation of the Heart

Bacq and Dworkin (1) produced no change in heart rate with single injections of pitressin in the cat immediately after total denervation, but observed a considerable acceleration of the chronically denervated heart.

Experiments were done on two animals after operative recovery from total cardiac denervation. Infusion with posterior pituitary extract produced no characteristic slowing of the heart rate in five experiments in our dogs, 1 to 10 days after total denervation (Table IV, Fig. 2). It caused the characteristic

TABLE IV

THE EFFECT OF POSTERIOR PITUITARY EXTRACT ON HEART RATE BEFORE AND AFTER COMPLETE DENERVATION OF THE HEART

Dog Barney			Dog Colonel		
Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate	Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate
Before denervation			Before denervation		
104	38		90	54	
90	36		90	42	
84	34		68	34	
102	40		62	31	
86	36	56			37
Both sympathetic chains removed, 1 vagus cut					
82	36				
88	48				
82	48	40			
Heart completely denervated			Heart completely denervated		
92	94		92	88	
96	102		82	78	
94	90	Nil			4

rise in blood pressure (Fig. 4C). Denervation did not alter the electrocardiogram from the normal, and infusion now made no change in it (Fig. 6).

Atropine alone or in combination with pituitary infusion had no effect on the heart or electrocardiogram (Fig. 6).

Discussion and Conclusions

Our results indicate that the inhibitory action of physiological doses of pituitary extract in the unanaesthetized dog is *solely* through the cardiac nerves. This is contrary to the opinion of several investigators who conclude that posterior pituitary extract acts, not only on the vagus mechanism, but also by direct myocardial depression, or coronary arterial constriction, or both.

Our experiments indicate that both sympathetic and vagus fibres are involved, since after vagotomy the extract is still able to cause some inhibition, but only so long as the sympathetic innervation is retained. Cardioinhibitory fibres have been demonstrated in the sympathetic supply to the dog heart (5); these are not affected by atropine. In the normal animal posterior pituitary inhibition is probably mainly through the vagus, since interruption of the sympathetic supply does not diminish it.

It is unlikely that the nervous inhibition is due to a carotid sinus-depressor reflex, since it may be initiated with no rise or only a slight rise in blood pressure (Table I).

Our experiments do not demonstrate whether the stimulation of inhibitory fibres is central or reflex from the heart. If reflex, afferent as well as efferent fibres must exist in both sympathetic and parasympathetic pathways.

We do not deny that posterior pituitary extract can cause coronary arterial constriction, by direct action, but this does not seem to be a primary factor in causing inhibition, for total denervation prevents the inhibitory effect of the extract. If constriction were to activate sensory receptors in the walls of the arteries, this might produce reflex inhibition which would be abolished by denervation.

Since total denervation abolishes the inhibitory effects, it is unlikely that posterior pituitary extract acts by myocardial depression. Support to this view is provided by the observation that the typical electrocardiographic signs that accompany the inhibition of the normal heart are abolished when the extract is infused after total cardiac denervation.

Our results with the dog are in partial agreement with those of Bacq and Dworkin with the cat, in which complete denervation abolished the inhibitory effect of pitressin. They found, however, that pitressin produced acceleration of the chronically denervated heart. This was never observed in our dogs either immediately after total denervation, or for two weeks thereafter.

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